The Metaphase Spindle in the Spermatogenetic Mitoses of Forficula Auricularia.

By

C. F. U. Meek, M.Sc., F.L.S., F.Z.S.

With Plate 17.

Introduction.

In a paper published at the beginning of this year I have shown that only one generalisation has been established concerning the mitotic spindle, viz. that it is not a figure formed entirely by the action of forces at its poles. The arguments put forward by Hartog in support of his "mitokinetic" force prove that, if this generalisation is denied, the spindle can be formed by no known forces. Gallardo and Rhumbler have been compelled to accept this proposition; and all early theories either admit it, or are disproved because they do not admit it.

I pointed out that in the circumstances we must collect data, and not attempt to explain mitosis until other generalisations have been established. Our knowledge of the morphological features of the cell and of the chemical nature of its component parts is rudimentary; and, since we know nothing concerning the forces working in mitosis and concerning the changes undergone by the mechanism in the course of evolution, speculation upon the subject is at present likely to prove abortive. The work of each decade has shown more and more that the cell is a highly complex entity; and the results of future research may prove it to be even more complex than we now suspect. Recently several writers upon both chromosomes and the achromatic portions of the cell have suggested that chemistry will provide the solution of problems that so far we have failed to solve. That eventually we must hand over to others problems that are found to lie beyond the scope of our investigations cannot be denied; but, until we have amassed data such that we can answer every question concerning the morphology and movements of structures that are visible in the cell, we are not justified in relying upon other branches of science for more than co-operation in our endeavour to explain these phenomena.

In a paper upon chromosome dimensions published last year I was able to show that increasing somatic complexity of the organism is accompanied by increase of the volume of chromatin in its germ-cell, and that the diameter of chromosomes becomes greater as we pass from low to higher phyla of the animal kingdom. I now propose to carry out similar investigations upon the phenomenon known as the mitotic spindle, and shall try to discover whether at a given moment in a given mitosis the length is constant or arbitrary; if it is found to be constant, we must determine the relationships between lengths at corresponding stages of successive mitoses, and must ask if these relationships are connected with those of other phenomena.

The stage that is most easy to identify in mitosis is the conclusion of the metaphase, when the chromosomes have undergone complete fission, and the daughter-rods, apposed to one another, are ready to move towards the two poles. I shall therefore make consideration of this stage the basis of my research, and shall determine the length of the spindle by measuring the distance between the centrosomes. I have chosen Forficula for these investigations, because the chromosomes are either spheres or very short rods; it is accordingly easy to recognise the moment at the conclusion of the metaphase when constriction is completed. Since the two centrosomes can seldom be brought into focus simultaneously at the highest magnifications, figures in which the spindle length is to be measured will be represented in the plates by two drawings: the first will show a lateral view of the equatorial plate at a magnification such that no doubt can exist concerning the stage of mitosis; the second will show a lateral view of the two centrosomes at a lower magnification, from which the length of the spindle must be deduced.

It is unlikely that these measurements will lead directly to an explanation of spindle formation; but they may suggest some further generalisation, which will bring us one step nearer to the solution of this problem.

Material and Methods.

The material was collected in July, and preserved in either Flemming's strong chromo-aceto-osmic acid fluid or the platino-aceto-osmic acid solution of Hermann. In an earlier paper I recommended the latter; but for these studies, in which extreme transparency of the cytoplasm is essential, the former has undoubtedly given the better results.

The testes were not dissected out until required for embedding; but the integument of the back was slit open to ensure immediate access of the fixative, in which the material remained for twenty-four or forty-eight hours. It was then washed in running water for twenty-four hours, and passed successively through 30 per cent., 50 per cent. and 70 per cent. aqueous solutions of alcohol, remaining for four hours in each of the two first-named and for eight hours in the last. It was then stored in a solution of 80 per cent. alcohol.

Later, the testes were placed in a 90 per cent. aqueous solution of alcohol for twenty-four hours, and then passed through a 95 per cent. solution, absolute alcohol, and xylol; after which they were embedded in paraffin having a melting-point of 52° C. Sections were cut 8 μ thick with an ordinary Cambridge rocking microtome, and were stained on the slide. The mordant used was an aqueous solution of iron alum, in which the sections remained for six hours; they were then stained for fifteen hours in Heidenhain's iron-hæmatoxylin, and the excess of colour was later washed out with a weak solution of the iron-alum. When a plasma stain was used in conjunction with the iron hæmatoxylin, the slides were first stained for ten minutes in eosin, care being taken that the colour was not destroyed by the strong solutions of alcohol in which they were later placed.

The preparations were studied by means of a Zeiss apochromatic oil-immersion objective of 2 mm. focus and N. A. 1·30, in conjunction with compensating oculars, Nos. 6, 12 and 18. When necessary, resolution was facilitated by interposing a Gifford screen. The source of illumination was an inverted incandescent gas-lamp, used in combination with the holoscopic oil-immersion substage condenser made by Messrs. Watson & Sons, of London. All drawings were made with a large Abbe camera lucida, and the magnification was estimated by means of a stage micrometer, graduated to read one hundredth of a millimetre. Whenever drawings were about to be made, both microscope platform and drawing table were carefully levelled; and error due to foreshortening was obviated by making drawings only of those spindles whose major axes lay exactly at right angles to the microscopic line of vision, i. e. whose centrosomes could be focussed simultaneously. Moreover, in order to minimise error due to draughtsmanship, the centrosomes of each spindle were drawn upon several occasions, and at least one hundred times in all.

The Length of the Mitotic Spindle at the Conclusion of the Secondary Spermatocyte Metaphase.

Figs. 1 to 5, Pl. 17, are drawings of polar views of the secondary spermatocyte metaphase, and all the chromosomes are shown. Those chromosomes that are short rods lie on the spindle in a manner such that their major axes are at right angles to the equatorial plane; consequently all must appear to be spherical in a perfectly polar view. The complex is composed of a single ring of ten chromosomes with two lying within it, or of a ring of nine chromosomes with three within it; the former arrangement is seen in figs. 1, 4 and 5, and the latter in figs. 2 and 3. I have failed to discover which arrangement is normal.

Fig. 6, Pl. 17, is a lateral view of the equatorial plate in a late secondary spermatocyte metaphase, and the bivalent chromosomes are seen to be undergoing constriction. This stage therefore immediately precedes that with which we propose to deal. The centrosomes of this cell are shown at a magnification of 889 diameters in fig. 49, Pl. 17, and the distance between them, estimated from this magnification, is 7·8 μ. Fig. 7, Pl. 17, shows the equatorial plate at the conclusion of the metaphase; constriction is here seen to be complete, and the dyads have become resolved into pairs of univalent chromosomes, which are ready to pass towards the two poles. Fig. 50, Pl. 17, shows the centrosomes of this cell, and the amount of their divergence is found from the drawing to be 8·1 μ. Figs. 8, 9 and 10, Pl. 17, are drawings of the equatorial plate in three cells undergoing successive stages of the anaphase, and the daughter-chromosomes are observed to be moving further and further apart. The centrosomes of these cells are represented in figs. 51, 52 and 53 respectively, and the distances between them, estimated from the known magnification, are 8·3, 8·5 and 8·7 μ.

Consideration of the drawings of these five cells suggests that the length of the spindle at the moment when constriction of the chromosomes is complete is a constant for this mitosis of the individual. It is reasonable to suppose that, if fixation had been delayed until the centrosomes of fig. 6 were 8·1 μ apart, the appearance of the chromosomes would have been similar in every respect to that seen in fig. 7; and we must likewise suppose that the equatorial plate in figs. 8, 9 and 10 was identical with that of fig. 7, when the length of the spindle in these three cells was 8·1 μ. We will, however, check these results by making drawings of a second set of five cells in which the distances between the centrosomes are respectively 7·8, 8·1, 8·3, 8·5 and 8·7 μ. Figs. 11 to 15, Pl. 17, are lateral views of the equatorial plate of these cells, and each is seen to correspond exactly with the drawing immediately above it. Constriction of the chromosomes is in progress in fig. 11, and is completed in fig. 12; in figs. 13, 14 and 15 the daughter-chromosomes appear to be moving further and further apart. The centrosomes of these cells are represented by figs. 54 to 58 respectively; and these drawings, made at a magnification of 889 diameters, are respectively identical with those given in figs. 49 to 53. These measurements, which have been made from cells belonging to the testes of a single specimen, can leave little doubt that the length of the spindle at the conclusion of the metaphase is a constant for this cell generation of the individual.

We must now ask if this constant may be assumed for all members of the species. Figs. 16 to 20, Pl. 17, are drawings of the equatorial plate of five cells in the testes of a second specimen, and each is seen to correspond exactly with the two drawings placed immediately above it. Fig. 16 shows a late metaphase, which is found to be concluded in fig. 17; figs. 18, 19 and 20 represent successive stages of the anaphase. Figs. 59 to 63, Pl. 17, show the centrosomes of these cells, drawn at a magnification of 889 diameters. And, since these drawings are respectively identical with figs. 49 to 53 and 54 to 58, we have reason for believing that the spindle length is a constant at the conclusion of the secondary spermatocyte metaphase in all specimens of F. auricularia.

The Length of the Mitotic Spindle at the Conclusion of the Primary Spermatocyte Metaphase.

Having discovered a probable constant for the spindle of the secondary spermatocyte metaphase, we will consider the primary spermatocyte mitosis. Figs. 21 to 24 represent polar views of the metaphase, and all the chromosomes are shown; fig. 25 is a drawing of the slightly later stage when the daughter-chromosomes have begun to move towards the poles. As in the secondary spermatocyte metaphase, the major axes of those chromosomes that are short rods are at right angles to the equatorial plane. The complex again appears to be composed of a ring of nine or ten chromosomes with three or two respectively lying within it; figs. 21, 22 and 25 represent the latter arrangement, and figs. 23 and 24 the former. I have again failed to discover which arrangement is normal.

Figs. 26 to 29, Pl. 17, are drawings of the equatoral plate of four cells of which the centrosomes, represented at a magnification of 889 diameters, are given in figs. 64 to 67, Pl. 17. The drawings on Pl. 17 clearly show that constriction of the tetrads has been completed, and that the daughter-chromosomes are ready to move apart. The length of the spindle, found from figs. 64 to 67, is without exception 10·4 μ; and, since the stage of the cells depicted is that with which we are dealing, we have reason for supposing that a constant exists also for this mitosis. The uneven pair of heterochromosomes is marked x in those cells in which it is visible.

Let us now measure the spindle length in four more cells in order to test the validity of this supposition. Figs. 30 to 33, Pl. 17, are drawings of the equatorial plate in cells of which the centrosomes are respectively represented by figs. 68 to 71, Pl. 17. Fig. 30 shows the constriction of the tetrads in progress; fig. 31 shows this constriction completed, as in figs. 26 to 29; and figs. 32 and 33 show the first divergence of the daughter-dyads. The distances between the poles of these cells, found from figs. 68 to 71, are respectively 10·2, 10·4, 10·7 and 10·9 μ, and therefore accord with the length of the spindle found for figs. 26 to 29. We must suppose that fig. 30 would have become identical with fig. 31, if fixation had not occurred until its centrosomes were 10·4 μ apart; and we must likewise suppose that the equatorial plates shown in figs. 32 and 33 passed through the stage shown in fig. 31, when their spindles were of the same length as that of the last named. In the circumstances I shall assume that the length of the spindle at the conclusion of the primary spermatocyte metaphase is a constant for this species.

I have found measurements in this metaphase more difficult to make than in that of the secondary spermatocyte; for spindles often appear to be distorted, and the distances between the poles may consequently be greater than those shown. The fact that no such distortion is observed in the spermatogonial and secondary spermatocyte mitoses suggests that the large size of the primary spermatocyte cells renders section-cutting, however carefully carried out, destructive of the true form of the spindle in certain cases. Such cells, however, may be recognised, because the spindle-fibres appear to be abnormally bent and twisted, and are often disconnected from the centrosomes. After careful consideration I am therefore satisfied that the measurements given represent accurately the dimensions within the cell.

The Length of the Mitotic Spindle at the Conclusion of the Secondary Spermatogonial Metaphase.

We will now consider the spermatogonial mitosis, and try to discover if a constant exists also for this metaphase. Figs. 34 to 37, Pl. 17, are drawings of polar views of this metaphase, showing all the chromosomes. Fig. 38 represents a polar view of one daughter-plate in the earliest anaphase. The complex appears to be composed of two concentric rings of fourteen and eight chromosomes respectively, while two chromosomes lie at the centre; this is the arrangement seen in figs. 34, 35, 37 and 38. In fig. 36, thirteen chromosomes constitute the outer ring, and nine the inner; but this arrangement may have resulted from abnormal movement caused by the process of section-cutting. The chromosomes that are short rods lie on the spindle in a manner such that their major axes are parallel to the equatorial plane, and thus differ in position from those of the spermatocyte metaphases.

Fig. 39, Pl. 17, is a drawing representing a lateral view of the equatorial plate before constriction of the bivalent chromosomes has begun. The centrosomes of this cell are shown in fig. 72, and the length of the spindle is found from this drawing to be 6·6 μ. Fig. 40 is a lateral view of the equatorial plate at a slightly later stage, when the dyads have begun to constrict; in this case the distance between the centrosomes is found from fig. 73 to be 6·9 μ. Fig. 41 represents the equatorial plate at the conclusion of the metaphase, and the dyads are seen to have become resolved into pairs of univalent spheres or rods, which are ready to move towards the poles. Fig. 74 shows the centrosomes of this cell, and the length of the spindle, estimated from the known magnification, is 7·1 μ. Figs. 42 and 43 are drawings of the equatorial plate in the earliest anaphase, and the daughter-chromosomes are seen to be moving apart. The centrosomes of these cells are represented in figs. 75 and 76 respectively, and their distances apart are 7·3 and 7·6 μ. It is difficult not to believe that figs. 39 and 40 would have resembled fig. 41, if fixation had not taken place until their spindle-poles were 7·1 μ apart; it is also difficult not to believe that figs. 42 and 43 were identical with fig. 41 at the moment when this was the distance between their poles.

Let us now turn to cells of this generation in the testes of another specimen. Fig. 44 shows the equatorial plate before constriction of the chromosomes has begun; figs. 45 and 46 show the equatorial plate while constriction is in progress; and figs. 47 and 48 show it at the conclusion of the metaphase, when constriction is completed. The centrosomes of these five cells are represented in figs. 77 to 81 respectively, and their distances apart accord with those already observed for this mitosis; for the length of the spindle is found to be 6·6 μ in fig. 44, 6·9 μ in figs. 45 and 46, and 7·1 μ in figs. 47 and 48. In the circumstances I shall assume that the length of the spindle at the conclusion of the metaphase is a constant for the secondary spermatogonial mitosis, as we have already assumed it to be for the primary and secondary spermatocyte.

The Ratios between the Lengths of the Mitotic Spindle at the Conclusion of the Spermatogonial and Spermatocyte Metaphases.

Having found that spindle lengths are probably constant at the conclusion of the metaphase of these mitoses, we must ask if the relationships between the lengths observed can be correlated with those of other phenomena.

Now the length of the spindle is evidently not connected with the volume of chromatin in the equatorial plane. I have shown in an earlier paper that the volume of chromatin in the metaphase is the same in the spermatogonial and primary spermatocyte mitoses; whereas it is reduced to one half in the secondary spermatocyte. If correlation is to be established, we must accordingly find the same spindle length in the metaphase of the two first-named mitoses, and a different spindle length in the last. We find, however, that the length of the spindle is not the same in any two of these metaphases. Furthermore, it is evident that the spindle length is not connected with the number of chromosomes present; for the number of chromosomes composing the spermatogonial complex is double that composing the complexes of the primary and secondary spermatocytes. The length of the spindle at the conclusion of the metaphase cannot therefore be correlated with the chromatin of the cell.

Let us now ask if the spindle length can be correlated with the cytoplasm. We know that no growth or resting stage occurs between the primary and secondary spermatocyte divisions, and that consequently the volume of the cell in the second must be half that in the first. Now the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other is 1·26 : 1·00. And this is almost exactly the ratio between the lengths of the spindle found for these two metaphases; for the lengths found are 10·4 and 8·1 μ, and—

$$10{\cdot}4 \ : \ 8{\cdot}1 \ :: \ 1{\cdot}28 \ : \ 1{\cdot}00.$$

If, therefore, inaccuracy of measurement is responsible for the slight difference between these ratios, the length of the spindle at the conclusion of each spermatocyte metaphase seems to be proportional to the radius of a sphere equal in volume to the cell.

We will now consider the secondary spermatogonial spindle. The length found for this mitosis at the stage with which we are dealing is 7·1 μ; and the ratio between this length and that observed at the corresponding stage of the primary spermatocyte is 1·00 : 1·46. Now we do not know the ratio between the volumes of the secondary spermatogonial and primary spermatocyte cells in the metaphase; for a long period of growth intervenes. But, if the spindle length at the conclusion of the spermatogonial metaphase is correlated with the cell volume, as we have reason for supposing that it is correlated in the two succeeding mitoses, we can determine the ratio between the volumes of the secondary spermatogonial and spermatocyte cells at this stage. Let us assume this. Now, the ratio between the radii of two spheres of which the volume of one is equal to three times that of the other is 1·44 : 1·00, and this is almost identical with the ratio between the lengths of the spindle at the conclusion of the primary spermatocyte and spermatogonial metaphases; for these lengths have been found to be 10·4 and 7·1μ, and we have already seen that—

10·4 : 7·1 : : 1·46 : 1·00

The difference between these two ratios is so small that it may be ignored; and, if our assumption concerning the relationship between the spindle length and cell volume in the metaphase is correct, we must realise that the volume of the primary spermatocyte cell at this stage is equal to three times that of the secondary spermatogonial. But the initial volume of the former must be half that of the latter, because the secondary spermatogonium divides to form two daughter primary spermatocytes. The volume of each primary spermatocyte must therefore be increased six-fold during the growth period. I have already shown by actual measurement that the volume of the chromatin is doubled during this period; and, since a large increase in the cell volume is always apparent at its conclusion, the possible connection between the length of the spindle and the volume of the cell in the spermatocyte metaphases may be extended to the spermatogonial mitosis.

Conclusion.

In the introduction of this paper I have said that the measurements to be made in the course of these investigations may suggest a further generalisation in the problem of mitosis. We have now made these measurements, and have found that they do suggest a new generalisation, viz. that the length of the mitotic spindle at the conclusion of the metaphase is proportional to the radius of a sphere equal in volume to the cell.

The measurements upon which this proposition is based have been made with great care; but it is possible that coincidence is responsible for the connection found between the spindle length and cell volume in the spermatocytes, and for the apparent connection in the spermatogonia. If, however, coincidence is not responsible, correlation is established between these phenomena in the spermatogenetic metaphases of this species. I intend in subsequent papers to measure spindle lengths at corresponding stages in other organisms; and, if the same relationships are found, I hope that my results will be corroborated by those of other cytologists.

Summary.

(1) The length of the mitotic spindle, i. e. the distance between the two centrosomes, at the stage of the metaphase when the chromosomes are undergoing constriction in the equatorial plane, appears to be a constant for each spermatogenetic mitosis of the species. The lengths found are 6·9, 10·2 and 7·8 μ for the secondary spermatogonia and primary and secondary spermatocytes respectively.

(2) The length of the mitotic spindle at the conclusion of the metaphase when the daughter-chromosomes are ready to move apart appears to be a constant for each spermatogenetic mitosis of the species. The lengths found are 7·1, 10·4 and 8·1 μ for the secondary spermatogonia and primary and secondary spermatocytes respectively.

(3) The length of the mitotic spindle in the earliest

anaphase, i. e. at the moment when the daughter-chromosomes have begun to move apart, appears to be a constant for each spermatogenetic mitosis of the species. The lengths found are 7·3, 10·7 and 8·3 μ for the secondary spermatogonia and primary and secondary spermatocytes respectively.

(4) The ratio between the lengths of the mitotic spindle at the conclusion of the primary and secondary spermatocyte metaphases is almost identical with the ratio between the radii of two spheres of which the volume of one is equal to twice that of the other; and the volume of the primary spermatocyte cell must be equal to twice that of the secondary spermatocyte at this stage, because no growth or resting stage intervenes.

(5) The ratio between the lengths of the mitotic spindle at the conclusion of the primary spermatocyte and secondary spermatogonial metaphases is almost identical with the ratio between the radii of two spheres of which the volume of one is equal to three times that of the other. The initial volume of the primary spermatocyte cell must be half that of the secondary spermatogonium, because the latter divides to form two daughter primary spermatocytes; but the large size of the last-named, observed at the close of the growth period, does not refute the suggestion that the initial volume is increased six-fold during this period.

(6) If coincidence is not responsible for the apparent connection between the ratios mentioned above, correlation is established between the cell volume and length of spindle in the spermatogenetic metaphases of this species.

March, 1913.

BIBLIOGRAPHY.

Gallardo, A.—"Les théories de la division cellulaire," 'Bull. de la Bib. Amer.,' No. 7 (1912).

Hartog, M.—"The New Force: Mitokinetism," 'Rep. Brit. Assoc., Sect. D.,' Sheffield (1910).

Meek, C. F. U.—"A Metrical Analysis of Chromosome Complexes, showing Correlation of Evolutionary Development and Chromatin Thread-width throughout the Animal Kingdom," 'Phil. Trans. Roy. Soc.,' ser. B, vol. cciii (1912).

Meek, C. F. U.—"The Correlation of Somatic Characters and Chromatin Rod-lengths, being a Further Study of Chromosome Dimensions," 'Journ. Linn. Soc. Zool.,' vol. xxxii (1912).

——— "The Problem of Mitosis," 'Quart. Journ. Micr. Sci.,' vol. 58 (1913).

Rhumbler, L.—"Die mechanische Erklärung der Ähnlichkeit zwischen magnetischen Kraftliniensystemen und Zellteilungsfiguren," 'Arch. f. Entwick.-mech.,' Bd. xvi (1903).

N.B.: A comprehensive list of publications dealing with mitotic phenomena is given in the bibliography of my paper, "The Problem of Mitosis."

EXPLANATION OF PLATE 17,

Illustrating Mr. C. F. U. Meek's paper, "The Metaphase Spindle in the Spermatogenetic Mitoses of Forficula Auricularia."

Figs. 1–4.—Polar views of secondary spermatocyte metaphase, showing all chromosomes. Specimen A.

Fig. 5.—Ditto. Specimen C.

Fig. 6.—Lateral view of equatorial plate in late secondary spermatocyte metaphase, showing constriction of bivalent chromosomes. Specimen A.

Fig. 7.—Lateral view of equatorial plate at conclusion of secondary spermatocyte metaphase, showing univalent daughter-chromosomes resulting from completed constriction of dyads. Specimen A.

Fig. 8.—Lateral view of equatorial plate in earliest secondary spermatocyte anaphase, showing divergence of daughter-chromosomes. Specimen A.

Fig. 9.—Ditto, later. Specimen A.

Fig. 10.—Ditto, later. Specimen A.

Fig. 11.—Lateral view of equatorial plate in late secondary spermatocyte metaphase, corresponding with fig. 6. Specimen A.

Fig. 12.—Lateral view of equatorial plate at conclusion of secondary spermatocyte metaphase, corresponding with fig. 7. Specimen A.

Fig. 13.—Lateral view of equatorial plate in earliest secondary spermatocyte anaphase, corresponding with fig. 8. Specimen A.

Fig. 14.—Ditto, later, corresponding with fig. 9. Specimen A.

Fig. 15.—Ditto, later, corresponding with fig. 10. Specimen A.

Fig. 16.—Lateral view of equatorial plate in late secondary spermatocyte metaphase, corresponding with figs. 6 and 11. Specimen B.

Fig. 17.—Lateral view of equatorial plate at conclusion of secondary spermatocyte metaphase, corresponding with figs. 7 and 12. Specimen B.

Fig. 18.—Lateral view of equatorial plate in earliest secondary spermatocyte anaphase, corresponding with figs. 8 and 13. Specimen B.

Fig. 19.—Ditto, later, corresponding with figs. 9 and 14. Specimen B.

Fig. 20.—Ditto, later, corresponding with figs. 10 and 15. Specimen B.

Fig. 21.—Polar view of primary spermatocyte metaphase, showing all chromosomes. Specimen D.

Figs. 22 to 24.—Ditto. Specimen A.

Fig. 25.—Polar view of equatorial plate in earliest primary spermatocyte anaphase. Specimen B.

Figs. 26–29.—Lateral views of equatorial plate at conclusion of primary spermatocyte metaphase, showing daughter-dyads resulting from completed constriction of tetrads. The unequal pair of heterochromosomes is marked *x*. Specimen A.

Fig. 30.—Lateral view of equatorial plate in late primary spermatocyte metaphase, showing constriction of tetrads. Specimen A.

Fig. 31.—Lateral view of equatorial plate at conclusion of primary spermatocyte metaphase, corresponding with figs 26–29. The unequal pair of heterochromosomes is marked *x*. Specimen A.

Fig. 32.—Lateral view of equatorial plate in earliest primary spermatocyte anaphase, showing divergence of daughter-dyads. Specimen A.

Fig. 33.—Ditto, later. Specimen A.

Figs. 34–37.—Polar views of spermatogonial metaphase, showing all chromosomes. Specimen D.

Fig. 38.—Polar view of earliest spermatogonial anaphase. Specimen B.

Fig. 39.—Lateral view of equatorial plate in early spermatogonial metaphase, before constriction of bivalent chromosomes has begun. Specimen D.

Fig. 40.—Lateral view of equatorial plate in late spermatogonial metaphase, showing constriction of dyads. Specimen D.

Fig. 41.—Lateral view of equatorial plate at conclusion of spermatogonial metaphase, showing univalent daughter-chromosomes resulting from completed constriction of dyads. Specimen D.

Fig. 42.—Lateral view of equatorial plate in earliest spermatogonial anaphase, showing divergence of daughter-chromosomes. Specimen D.

Fig. 43.—Ditto, later. Specimen D.

Fig. 44.—Lateral view of equatorial plate in earliest spermatogonial metaphase, corresponding with fig. 39. Specimen B.

Figs. 45, 46.—Lateral view of equatorial plate in late spermatogonial metaphase, corresponding with fig. 40.

Figs. 47, 48.—Lateral view of equatorial plate at conclusion of spermatogonial metaphase, corresponding with fig. 41. Specimen B.

Fig. 49.—Centrosomes belonging to equatorial plate depicted in fig 6. Estimated divergence in cell, 7·8 μ.

Fig. 50.—Ditto in fig. 7. Ditto, 8·1 μ.

Fig. 51.—Ditto in fig. 8. Ditto, 8·3 μ.

Fig. 52.—Ditto in fig. 9. Ditto, 8·5 μ.

Fig. 53.—Ditto in fig. 10. Ditto, 8·7 μ.

Fig. 54.—Ditto in fig. 11. Ditto, 7·8 μ.

Fig. 55.—Ditto in fig. 12. Ditto, 8·1 μ.

Fig. 56.—Ditto in fig. 13. Ditto, 8·3 μ.

Fig. 57.—Ditto in fig. 14. Ditto, 8·5 μ.

Fig. 58.—Ditto in fig. 15. Ditto, 8·7 μ.

Fig. 59.—Ditto in fig. 16. Ditto, 7·8 μ.

Fig. 60.—Ditto in fig. 17. Ditto, 8·1 μ.

Fig. 61.—Ditto in fig. 18. Ditto, 8·3 μ.

Fig. 62.—Ditto in fig. 19. Ditto, 8·5 μ.

Fig. 63.—Ditto in fig. 20. Ditto, 8·7 μ.

Figs. 64–67.—Ditto in figs. 26–29 respectively. Ditto, 10·4 μ.

Fig. 68.—Ditto in fig. 30. Ditto, 10·2 μ.

Fig. 69.—Ditto in fig. 31. Ditto, 10·4 μ.

Fig. 70.—Ditto in fig. 32. Ditto, 10·7 μ.

Fig. 71.—Ditto in fig. 33. Ditto, 10·9 μ.

Fig. 72.—Ditto in fig. 39. Ditto, 6·6 μ.

Fig. 73.—Ditto in fig. 40. Ditto, 6·9 μ.

Fig. 74.—Ditto in fig. 41. Ditto, 7·1 μ.

Fig. 75.—Ditto in fig. 42. Ditto, 7·3 μ.

Fig. 76.—Ditto in fig. 43. Ditto, 7·6 μ.

Fig. 77.—Ditto in fig. 44. Ditto, 6·6 μ.

Figs. 78, 79.—Ditto in figs. 45 and 46. Ditto, 6·9 μ.

Figs. 80, 81.—Ditto in figs. 47 and 48. Ditto, 7·1 μ.

Fig. 82.—Divisions of stage micrometer, 10 μ apart, drawn at same magnification as figs. 49–81. The magnification of these figures is estimated from this to be 889 diameters.

N.B.: Specimen A—preserved in fixative of Flemming, and stained with iron-hæmatoxylin. Specimen B—preserved in fixative of Hermann and stained with iron-hæmatoxylin. Specimen C—preserved in fixative of Flemming, and stained with iron-hæmatoxylin and eosin. Specimen D—preserved in fixative of Flemming and stained with iron-hæmatoxylin.